

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 513 922 A1**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 92201359.4

(51) Int. Cl.<sup>5</sup>: **A23L 3/3463**, A23D 7/00,  
A23C 19/11, A61K 7/48,  
A01N 25/04

(22) Date of filing: 12.05.92

(30) Priority: 17.05.91 EP 91204210

(43) Date of publication of application:  
19.11.92 Bulletin 92/47

(94) Designated Contracting States:  
AT BE CH DE DK ES FR GB GR IT LI LU MC  
NL PT SE

(71) Applicant: **GIST-BROCADES N.V.**  
Wateringseweg 1 P.O. Box 1  
NL-2600 MA Delft(NL)

(72) Inventor: **Tan, Hong Sheng**  
Buizerdstraat 94  
NL-2665 TG Bleiswijk(NL)  
Inventor: **van Rijn, Ferdinand Theodorus**  
Jozef  
Valkenlaan 7  
NL-2623 HM Delft(NL)  
Inventor: **Dijkgraaf, Bernardus Leonardus**  
Johannes  
Boomkleverstraat 52  
NL-2623 GW Delft(NL)  
Inventor: **de Haan, Ben Rudolf**  
Wilgendreef 95  
NL-2272 EN Voorburg(NL)

(54) Coating for foods and agricultural products to prevent the growth of moulds.

(57) An oil in water emulsion is provided which comprises a suitable emulsifier, optionally a suitable co-emulsifier, and at least one suitable antifungal agent, whereby the antifungal agent is in suspension in the waterphase.

EP 0 513 922 A1

## FIELD OF THE INVENTION

The present invention relates to antifungal preparations to be used on foods, such as cheese or sausages; or on agricultural products, such as fruit, vegetables, tubers and flower bulbs; or for pharmaceutical or cosmetic treatment; and to a method for treating these foods and agricultural products with such preparations; and to foods and agricultural products so treated. The invention relates particularly to oil in water emulsions containing a suitable antifungal agent and to methods for the preparation of such emulsions and to a method for controlled release of such antifungal agent from such emulsion.

## BACKGROUND OF THE INVENTION

For more than 20 years natamycin has been used to prevent growth of moulds on cheese and sausages. References are:

1. Daamen, C.B.G. and Berg, G van den; "Prevention of mould growth on cheese by means of natamycin." Voedingsmiddelentechnologie, 18 (2), 26-29, (1985).
2. Oostendorp, J.G.; "Natamycin", Antonie van Leeuwenhoek, 47 (2), 170-171, (1981)
3. Neviani, E.; Emaldi, G.C.; Carini, S.; "Use of pimaricin as a fungicide on cheese rind: technology and the effects on the cheese surface flora." Latte, 6 (5), 335-343, (1981)
4. Holley, R.A.; "Prevention of surface mould growth on Italian dry sausage by natamycin and potassium sorbate." Applied and Environmental Microbiology, 41 (2), 422-429, (1981)
5. Morris, H.A.; Castberg, H.B.; "Control of surface growth on Blue cheese using pimaricin". Cultured Dairy Products Journal, 15 (2), 21-23, (1980)
6. Baldini, P.; Palmia, F.; Raczyński, R.G.; Campanini, M.; "Use of pimaricin for preventing mould growth on Italian cured meat products." Industria Conserve, 54 (4), 305-307, (1979)
7. Lueck, H.; Cheesman, C.E.; "Mould growth on cheese as influenced by pimaricin (natamycin) or sorbate treatments." South African Journal of Dairy Technology, 10 (3), 143-146, (1978)

It is known in the art, that e.g. cheeses are treated by immersion in a suspension of natamycin in water or covered by an emulsion of plastic in water, (mostly polyvinylacetate). Sausages are mainly treated by immersion or by spray coating with a suspension of natamycin in water.

A disadvantage of immersion is the necessity of a large scale preparation of immersion liquid. This means a considerable spillage of immersion liquid, as in most cases the left over fluid cannot be re-used due to "pollution" by cheese ingredients and the limited stability of the liquid caused by bacterial contamination. This means non-economical use of the preparation and causes an environmental burden.

Also, fatty surfaces like the surface of a GRANA type of cheese or of sausages cannot be covered homogeneously, which means there is no optimal protection against the growth of moulds. Addition of a surface active agent (like sodium lauryl sulphate or a polyoxyethylene ester) will be useful in some cases, but mostly the effect is insufficient and/or such treatment is yielding a sticky surface.

Natamycin containing plastic coatings are applied on hard cheeses like the "Gouda" type. Unlike immersion, plastic coating of cheese has the advantage that the surface of the cheese can be treated by spunging the emulsion, so there is no contaminated left over. Applying plastic coating is economically attractive as the coating reduces loss of moisture and does not negatively effect the development of taste. Reference:

8. Ruig, W.G.; Berg, G. van den; "Influence of the fungicides sorbate and natamycin in cheese coatings on the quality of the cheese." Netherlands Milk and Dairy Journal, 39 (3), 165-172, (1985)

Besides, a plastic coating gives a cheese a nice, glossy appearance. In cheese handling, conservation by means of natamycin containing plastic coating has become a common practice because of extremely good results. However, it is a definite disadvantage, that these coatings cannot be considered as edible and thus have to be removed from the surface of the cheese prior to consumption. In practice it is not possible just to remove the coating: Part of the cheese will go with it. Also, because of being unfit for consumption, coating should not be used on soft cheese as the cheese rind is not removed. Besides, coating of soft cheese causes an unnatural appearance.

Further, spray coating is very difficult due to frequent blockage of spray-nozzles. Still another disadvantage is the slow drying of the coating. In practice, first one side of the cheese is treated and after standing overnight for drying, the other side is covered with the coating.

Normally, in the initial period of storage, cheese or sausage must be treated with natamycin several times, the reason being, that in the initial period cheese and sausage are still very moist and therefore very sensitive to contamination by moulds.

For application on foods and especially for application on cheese, in case plastic coating is not

desirable, the quality of the composition has to meet specific requirements.

Firstly, the maintenance of a constant level of active natamycin, sufficient to prevent the growth of moulds over a considerable period of time.

Secondly, for foods, the ingredients in the composition should conform to "the E numbers classification" (official admission as food additive by the European Community, as established by the pertaining Food Additive Directives of the European Commission).

Furthermore, after application the coating should be strong enough to prevent damage due to handling and the "cosmetic" aspect of the coating should be highly acceptable.

Emulsions of the oil in water type are formulations with several advantages: concentrates can easily be diluted with water to any desired concentration and application may occur in several convenient manners e.g. for cheese by spreading the emulsion with a sponge, by immersion of the cheese in the emulsion or by applying the emulsion by means of spraying device.

It is generally accepted that the release of an active ingredient from a medium depends to a considerable extent of the composition of the carrier in which such active ingredient is contained as well as of the diffusion rate of the active ingredient itself.

#### SUMMARY OF THE INVENTION

This invention provides oil in water emulsions suitable for use as edible antifungal coating for foods, such as cheese or sausages. Also the invention provides oil in water emulsions suitable as an antifungal coating for agricultural products like fruits, vegetables, tubers and flower bulbs. Equally, the invention provides oil in water emulsions suitable for pharmaceutical or cosmetic treatment of human or animal skin. These emulsions can be provided in various ways. A preferred embodiment is a natamycin emulsion concentrate that can be diluted with water by the user to any desired concentration. Also the emulsion may be provided as a blank base in order to incorporate natamycin in any desired concentration directly before use.

Further, this invention provides methods for preparation of such emulsions. And a method for treating foods and agricultural products with such emulsions. And foods and agricultural products so treated.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the principle of the effect of controlled release of an active component on the time of action of such component (e.g. natamycin).

The solubility of natamycin was supposed to be 50 ppm. At the beginning the solution was "over"-saturated, starting with a dissolved amount of natamycin higher than the solubility value of 50 ppm.

Curve (1) illustrates the situation at a concentration of about 100 ppm of natamycin. By elimination processes the dissolved amount of natamycin will decrease gradually until it drops beneath the effective concentration (about 10 ppm).

Curve (2) illustrates the effect of increased natamycin concentration in suspension. At first the dissolved amount will decrease in the same way as in case 1. However, the presence of the solid undissolved natamycin in the suspension will serve as a depot. When the dissolved amount drops to a value of about 50 ppm this value will be maintained until all the solid natamycin has been dissolved. Then the concentration will gradually decrease and drop beneath the effective concentration. The total effect is an extended effectivity.

Curve (3) illustrates what will happen with a controlled released natamycin. Provided that the release is such that the dissolved concentration of natamycin is kept between the effective concentration and the concentration at saturation. With the same amount of natamycin in suspension as in case (2), the time of action will be further extended. The area under curve (3) must be equal to the area under curve (2).

Figures 2, 3, 4 and 5 show the mould inhibiting properties of various natamycin compositions at values of 100 ppm, 200 ppm and 500 ppm of natamycin in suspension respectively. The compositions A and B are specified in Table 1 (page 16).

#### DETAILS OF THE INVENTION

The emulsion according to this invention is one that contains an antifungal agent, e.g. natamycin. Alternatively, the antifungal agent may be added to the emulsion just before use. Instead of natamycin, or in combination with natamycin, other suitable antifungal compounds such as Imazalil, Lucensomycin, Amphotericin B or Nystatin may be applied.

Further the emulsion contains water, a suitable emulsifier, solid fats, and optionally a co-emulsifier, a thickening agent, a buffering system, and a preservative.

Emulsifiers are a variety of substances, able to create a link between fats and water, due to their hydrophilic as well as their lipophilic properties. Especially suitable for emulsification are polyethylene alkyl ethers, e.g. Cetomacrogol® 1000, polyoxyethylene sorbitan fatty acid esters, e.g. Tween® 60, 61 and 65, and/or anionic tensides, e.g. sodium lauryl sulphate and the dairy-related sodium caseinate or whey protein. The emulsifying agent in a ready to use emulsion is used in amounts of 0.1 - 15%, preferably 0.25 - 5.0%, and more preferably 0.25 - 1.0%. Accordingly, amounts of emulsifier in a concentrated emulsion can vary between 0.5 and 30%, more preferably between 2.5 and 20%.

The solid fats used are preferably edible fats, at least when used in food applications. Suitable fats are triglycerides on a base of coconut- or palm kernel oil, e.g. compositions known under the brand names of Witocan®, Witepsol® and Toffix® (Dynamite Nobel), concentrates of butter fat or similar products.

After emulsification these fats form the solid phase in the preparation. A very useful property of these fats is the narrow melting range, e.g. for Toffix® 34 - 36 °C. Melting points may vary between 30 and 60 °C, preferably between 35 and 45 °C. An advantage of such preparations is the fact that, if necessary, more viscous preparations can easily be liquified by slightly warming up to a relatively low temperature. Fats in a ready to use emulsion are used in amounts of 1 - 25%, preferably 3 - 10%. Amounts of fat in a concentrated emulsions may vary between 10% and 60%.

Co-emulsifiers are used to improve the physical stability of the emulsion and also to improve the "cosmetic aspect" of the coating. Co-emulsifiers may be in amounts up to 20%, preferably 2 - 10%. A very suitable co-emulsifier is a sucrose-ester, e.g. sucrose ester of palm kernel oil known under the brand name of Celynol® (Rhône Poulenc). Other suitable co-emulsifiers are mono- and diglycerides of fatty acids or mixtures thereof, e.g. types known under the brand name of Imwitor® (Dynamite Nobel).

Emulsions of a sugar ester and a fatty acid are described in French patent 2 371 149. That patent provides a composition of predominantly sucroglycerides as an emulsion for coating of fruits in order to slow down the ripening process. No mold inhibitors or other active substances are used.

In the composition according to the present invention such sucroglycerides are only used as, optional, co-emulsifiers.

Suitable thickening agents are for instance gelatin, chitosan, pectin, alginates, tragacanth, and modified cellulose (e.g. hydroxypropyl cellulose or carboxymethyl cellulose). These agents may be used in amounts of 1 - 10%.

Optionally a buffering system may be added to stabilize the pH of the emulsion on a value of approximately 4.5. A suitable buffering system is for instance a mixture of citric acid and trisodium citrate. A preservative is added to prevent bacterial growth in the emulsion. A suitable preservative is e.g. sorbic acid. Optionally glycerol may also be added to enhance the firmness of the coating after application and drying of the emulsion. Suitable amounts vary from 1 to 5%.

The invention is particularly suitable for the formulation of natamycin and provides an excellent antifungal coating after application on food and on agricultural products. Natamycin is only active when it is in an available phase i.e. in solution. In aqueous systems the solubility of natamycin ranges from 30 - 50 ppm (parts per million), depending of the presence of other substances. However, in order to be effective against fungus growth the minimal inhibitory concentration is less than 10 ppm. Accordingly, at least 3 - 5 times the concentration needed would usually be available in solution.

Moreover, natamycin in solution is susceptible to inactivation by degradation (e.g. by oxidation, hydrolysis, photolysis). By adapting the concentration to the minimal inhibitory level, the inactivation is minimized and the time of effective activity of the active ingredient will be extended. In the composition according to the invention, the antifungal substance is released and maintained in solution at a minimal inhibitory concentration, thereby considerably enhancing and extending the effective antifungal activity of the composition. This is demonstrated in Figure 1. The dissolution rate was measured in a two-compartment cell separated by a membrane. The composition of the invention was put in one compartment, while a flow of solvent was maintained in the other cell, as a consequence of which the antifungal substance by diffusion through the membrane was "extracted" from the composition. Concentration of the dissolved antifungal was determined by methods known in the art.

The composition according to the invention may be prepared by the usual methods for emulsifying systems. E.g.: fats and fat soluble ingredients including sorbic acid are melted at a temperature of 70 - 80 °C. Water soluble ingredients are dissolved in the water, this solution is also heated to a temperature of 70 - 80 °C and the active ingredient (e.g. natamycin) is suspended in the hot solution. Both liquids are thoroughly mixed by means of a Turrax® mixer. After mixing, the emulsion is brought to ambient temperature by forced cooling and slow stirring to prevent foaming. Optionally the active ingredient may be

added to the cooled emulsion, e.g. just before application.

The following examples will serve as illustration and should not be considered as a limitation of the invention.

5 Example 1

Toffix® P (50 g) and Celydol F1® (5 g) were melted together at a temperature of 70 - 80°C.

Cetomacrogol® 1000 (5 g) was dissolved in water (940 g). This solution was heated to 70 - 80°C and then added to the melt. Subsequently the mass was thoroughly mixed with a Turrax® mixer during 5 minutes. After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

The result was a low viscous milky liquid.

15 Example 2

The same procedure as described in Example 1 was followed, except that before heating to 70 - 80°C gelatin (5 g) was also added to the water phase.

The result was a low viscous milky liquid.

20 Example 3

The procedures of Example 1 was repeated, except that before heating to 70 - 80°C Klucel® LF, carboxymethyl cellulose, (5 g) was also added to the water phase.

The result was a low viscous milky liquid.

25 Example 4

The procedures of Example 1 was repeated, except that an optional amount of natamycin (e.g. 8 g) was added to the hot water phase before mixing with the hot melt.

30 The result was a low viscous milky liquid.

Example 5

35 The procedure of Example 2 was repeated, except that 8 g of natamycin was added to the hot water phase before mixing with the hot melt.

The result was a low viscous milky liquid.

Example 6

40 The procedure of Example 3 was repeated, except that 8 g of natamycin was added to the hot water phase before mixing with the hot melt.

The result was a low viscous milky liquid.

Example 7

45 Witocan® 42/44 (50 g) and Celydol® F1 (5 g) were melted together at a temperature of 70 - 80°C.

Cetomacrogol® 1000 (5 g) was dissolved in water (940 g). This solution was heated to 70 - 80°C and then added to the melt. Subsequently the mass was thoroughly mixed with a Turrax® mixer during 5 minutes. After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming

50 The result was a low viscous milky liquid.

Example 8

55 The procedure of Example 7 was repeated, except that an optional amount of natamycin (e.g. 8 g) was added to the hot water phase before mixing with the hot melt.

The result was a low viscous milky liquid.

#### Example 9

The procedure of Example 7 was followed, but before heating to 70 - 80 °C gelatin (5 g) was added to the water phase. Furthermore, 8 g of natamycin was added to the hot water phase before mixing with the hot melt.

The result was a low viscous milky liquid.

#### Example 10

Witepsol® S55 (50 g) and Celynol® F1 (5 g) were melted together at a temperature of 70 - 80 °C.

Cetomacrogol® 1000 (5 g) and carboxymethyl cellulose (5 g) were dissolved in water (940 g).

This solution was heated to 70 - 80 °C and then added to the melt. Subsequently the mass was thoroughly mixed with a Turrax® mixer during 5 minutes. After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

The result was a low viscous milky liquid.

#### Example 11

The procedure of Example 10 was repeated, except that an optional amount of natamycin (e.g. 8 g) was added to the hot water phase before mixing with the hot melt.

The result was a low viscous milky liquid.

#### Example 12

Toffix® P (50 g), Celynol® F1 (5 g) and sorbic acid (5 g) were melted together at a temperature of 70 - 80 °C.

Cetomacrogol® 1000 (10 g), citric acid monohydrate (1.16 g) and trisodiumcitrate dihydrate (1.84 g) were dissolved in water (940 g).

This solution was heated to 70 - 80 °C and then natamycin (8 g) was suspended in the warm solution. The natamycin suspension was added to the melt and subsequently thoroughly mixed with a Turrax® mixer during 5 minutes.

After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

The result was a low viscous milky liquid.

#### Example 13

The procedure of Example 12 was repeated except that the solid fat Toffix® P was replaced by Witocan® 42/44 (50 g).

The result was a low viscous milky liquid.

#### Example 14

The components and the manufacturing procedure were the same as described in Example 12, however the resulting emulsion was a concentrated emulsion. The amounts in the emulsion were:

- Toffix® P (250 g), Celynol® F1 (25 g) and sorbic acid (5 g)
- Cetomacrogol® 1000 (50 g), citric acid monohydrate (5.8 g) and trisodiumcitrate (9.2 g)
- natamycin (40 g)
- water (640 g)

The result was a moderately viscous milky liquid.

#### Example 15

The procedure according to Example 14 was repeated, but instead of Cetomacrogol® 1000, Tween® 61 (50 g) was used as the emulsifier. Tween® 61 is polyoxyethylene 4 sorbitan monostearate.

The result was a moderately viscous milky liquid.

Example 16

The procedure according to Example 14 was repeated, but instead of Cetomacrogol® 1000, sodium lauryl sulphate (25 g) was used as the emulsifying agent.

5 The result was an emulsion with a creamy appearance.

Example 17

10 A concentrate of butter fat (250 g), Celydol® F1 (25 g) and sorbic acid (5 g) were melted together at a temperature of 70 - 80 °C. Cetomacrogol® 1000 (50 g), citric acid monohydrate (5.8 g) and trisodiumcitrate dihydrate (9.2 g) were dissolved in water (640 g). This solution was heated to 70 - 80 °C and then natamycin (40 g) was suspended in the warm solution.

The natamycin suspension was added to the melt and subsequently thoroughly mixed with a Turrax® mixer during 5 minutes.

15 After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

The result was a moderately viscous milky liquid.

Example 18

20 The procedure according to Example 17 was repeated, but instead of Cetomacrogol® 1000, Tween® 65 (50 g) was used as the emulsifier. Tween® 65 is polyoxyethylene 20 sorbitan tristearate.

The result was an emulsion with creamy appearance.

25 Example 19

Toffix® P (100g), Celydol® F1 (5 g) and sorbic acid (5 g) were melted together at a temperature of 70 - 80 °C.

30 Sodiumcaseinate (100 g) was dissolved in water (780 g). This solution was heated to 70 - 80 °C and then natamycin (8 g) was suspended in the warm solution.

The natamycin suspension was added to the melt and subsequently the mass was thoroughly mixed with a Turrax® mixer during 5 minutes.

After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

35 The result was a moderately viscous milky liquid.

Example 20

40 Witexsol® S55 (50 g) and Imwitor® 742 (5 g) were melted together at a temperature of 70 - 80 °C.

Cetomacrogol® 1000 (5 g) was dissolved in water (940 g). This solution was heated to 70 - 80 °C and then added to the melt. Subsequently, the mass was thoroughly mixed with a Turrax® mixer during 5 minutes. After that the emulsion was cooled down (forced cooling) to ambient temperature. During the cooling time the emulsion was stirred slowly without drawing a vortex to prevent foaming.

The result was a low viscous milky liquid.

45

Example 21

50 A concentrate of butter fat (250 g) was melted at a temperature of 60-70 °C. Whey protein (260 g), glycerol (50 g) and potassium sorbate (5 g) were dissolved in water (427 g). This suspension was heated to 60-70 °C and natamycin (8 g) was added to the warm solution. The melted butter fat was then added to the warm aqueous suspension and subsequently thoroughly mixed during 5 minutes by means of a Turrax® mixer.

After that the emulsion was cooled down to ambient temperature by forced cooling. During cooling the mass was stirred slowly without drawing a vortex to prevent foam formation.

55 The result was a pourable yellowish viscous liquid.

Example 22

In the following experiment the effect on mould prevention on the surface of cheese of a composition according to the invention was compared to that of plasticcoat and an aqueous suspension of natamycin.

A freshly brined wheel of Gouda cheese was cut horizontally into two parts. Each part was then cut into pieces of 5 by 5 by 5 cm. Only pieces with a flat original surface were taken for the experiment.

5 The original surface area (the rind) was then inoculated with about  $10^5$  CFU of a mixture of equal amounts of spores of *Aspergillus versicolor*, *Penicillium verrucosum* var. *verrucosum*, and *Penicillium verrucosum* var. *cyclopium*. The inoculation was performed by applying 0.15 ml of a spore suspension containing about  $10^6$  CFU/ml to the surface of the pieces of cheese.

10 After 12-hours' incubation at about 6°C, the pieces of cheese were treated with the different compositions. For each treatment 10 pieces of cheese were taken. The treatment was performed by immersion of the pieces of cheese one by one into the parent liquid compositions. For this purpose each liquid composition was put into a beaker of 1000 ml. To make the viscous plastic emulsion suitable as an immersion liquid it was first diluted by mixing 2 parts of it with 3 parts of water.

15 By weighing of the beaker containing the dipping liquid before and after the immersion of the pieces of cheese the amount of liquid applied to the cheese can be determined. With the known surface area of the pieces of cheese and the known concentration of the natamycin the amount of natamycin applied can be calculated. After treatment the pieces of cheese were incubated at 15°C and at a relative humidity of 95%.

20 The results are summarized in Figures 2, 3, 4 and 5. Composition A and Composition B, as indicated in the figures, are compositions according to the invention. Natamycin contents of these compositions and natamycin applied per dm<sup>2</sup> of cheese surface are shown in Table 1.

Table 1

25 The amount of natamycin applied to the cheese surface by dipping into several compositions containing 100, 200 and 500 ppm of natamycin in suspension respectively (mg/dm<sup>2</sup>).

	Aqueous suspension	Diluted plasticcoat	Composition A	Composition B
100 ppm	0.06	0.12	0.09	----
200 ppm	0.13	0.23	0.17	0.19
500 ppm	0.33	0.57	0.42	----

40 The basic compositions of A and B are as follows:

	A		B	
45 Toffix® P	50	g	50	g
Celysol® F1	5	g	5	g
Cetomacrogol® 1000	5	g	--	
Tween® 60	--		5	g
Citric acid mono hydrate	1.16	g	1.16	g
50 Trisodium citrate dihydrate	1.84	g	1.84	g
Sorbic acid	5	g	5	g
Water	940	g	940	g

55 The treatment with a composition according to the invention resulted in a considerably more effective suppression of mould growth than a similar treatment by plasticcoat or by a plain aqueous suspension with similar contents of natamycin. This result is the more surprising in considering the amounts of natamycin



actually applied to the cheese.

### Example 23

- 5 This example demonstrates the effect on the amounts of bloomers of flowerbulbs when treated with a composition according to the invention as compared to that of a treatment with an aqueous suspension of natamycin. 700 Flower bulbs (*Lilium longiflorum*) were divided into 35 portions of 20 bulbs each. 15 Portions were inoculated by immersion in a spore suspension containing  $2 \times 10^5$  CFU/ml of a mixture of *Penicillium* species. These species were obtained by cultivation of spores isolated from bulbs contaminated with green mold. The bulbs were covered with plastic sheets and then stored at 20 °C in the usual manner. 10 After one week of incubation the bulbs were treated according to the following scheme.

Item Nr.	Inoculated	Treatment with
15 1A to 1E	no	Nothing
2A to 2E	no	Composition C
3A to 3E	no	Composition D
4A to 4E	no	Nothing
5A to 5E	yes	Nothing
20 6A to 6E	yes	Composition C
7A to 7E	yes	Composition D

Thus each treatment consisted of 5 items of 20 bulbs. The flower bulbs were treated by means of immersion in the parent liquid compositions.

25 Composition C is a 4:1 (w/w) mixture of water and the composition according to example 14. Composition C thus contained 0.8% of natamycin.

Composition D is a suspension of 0.8% of natamycin in water.

After the treatments the bulbs were successively stored 2 weeks at 20 °C and 8 weeks at 2 °C. Then the bulbs were planted in a glass house and after 6 months of growing the items were assessed on the amount of blooming plants.

The results are summarized in table 2.

Table 2

Percentage of bloomers						
Treatment Nr	A	B	C	D	E	Average
35 1	60	45	45	45	35	46.0 ± 8.9
40 2	85	60	100	80	80	81.0 ± 14.3
3 3	50	70	45	55	40	52.0 ± 11.5
4 4	55	70	30	55	30	48.0 ± 17.5
5 5	45	25	55	20	30	35.0 ± 14.6
45 6	85	60	70	80	70	73.0 ± 9.7
7 7	55	45	60	40	45	49.0 ± 8.2

The table showed that a treatment with a composition according to the invention (2 and 6) gave better results than no treatment (1, 4 and 5) or a comparable treatment without such a composition (3 and 7).

### Claims

1. An oil in water emulsion, which comprises a suitable emulsifier, optionally a suitable co-emulsifier, and at least one suitable antifungal agent, whereby the antifungal agent is in suspension in the waterphase.
2. An oil in water emulsion according to claim 1, characterized in that the antifungal agent is natamycin.
3. An oil in water emulsion according to claim 1, characterized in that the oil phase of the emulsion is

composed of fats, which are solid at ambient temperatures, preferably fats with a melting range between 30 °C and 60 °C, more preferably triglycerides.

4. An oil in water emulsion according to claim 1, characterized in that the emulsifier used is selected from a group consisting of a polyethylene alkyl ether and an anionic tenside.
5. An oil in water emulsion according to claim 1, characterized in that the co-emulsifier used is selected from a group consisting of a sucrose ester, and a mono- or diglyceride of a fatty acid.
6. An oil in water emulsion according to claim 1, wherein the combination of the emulsifier and co-emulsifier is chosen so as to obtain a reduced release of the suspended antifungal agent in the waterphase of the emulsion, in comparison to its normal solubility in water.
7. A process for making emulsions according to any one of claims 1 - 6, characterized in that suitable solid fats are melted at elevated temperatures optionally with the suitable co-emulsifier, whereupon an aqueous solution of the emulsifier, and or thickening agents and the buffer, heated at an elevated temperature is added to the melt, optionally with the antifungal agent suspended in the aqueous solution or added afterwards.
8. A process for inhibiting or suppressing superficial growth of moulds or fungi on the surface of foods or agricultural products or on human or animal skin by treating the surface of such foods or agricultural products or treating human or animal skin with the emulsion according to any one of claims 1 - 6.
9. Foods or agricultural products treated according to claim 8.

Fig. 1

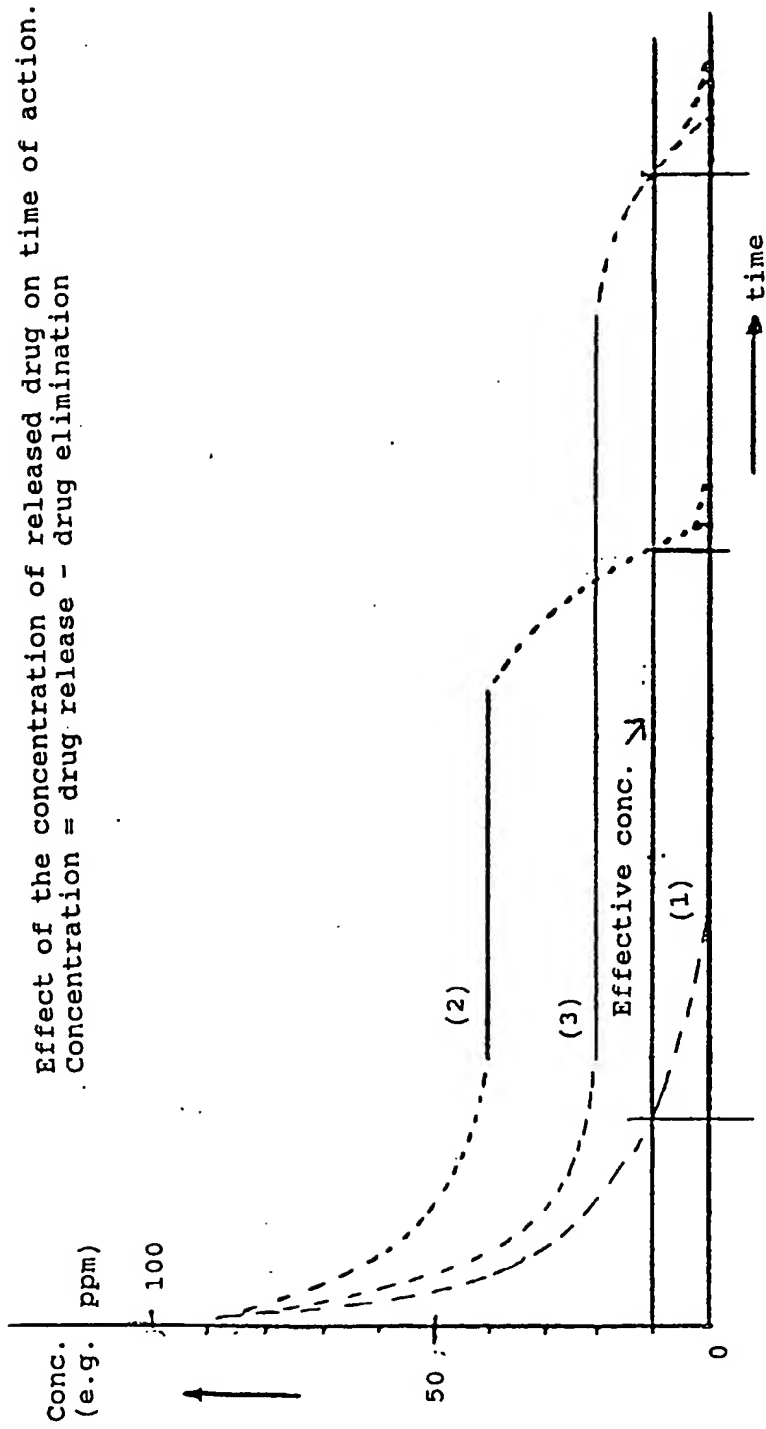


Fig. 2

## 100 PPM OF NATAMYCIN

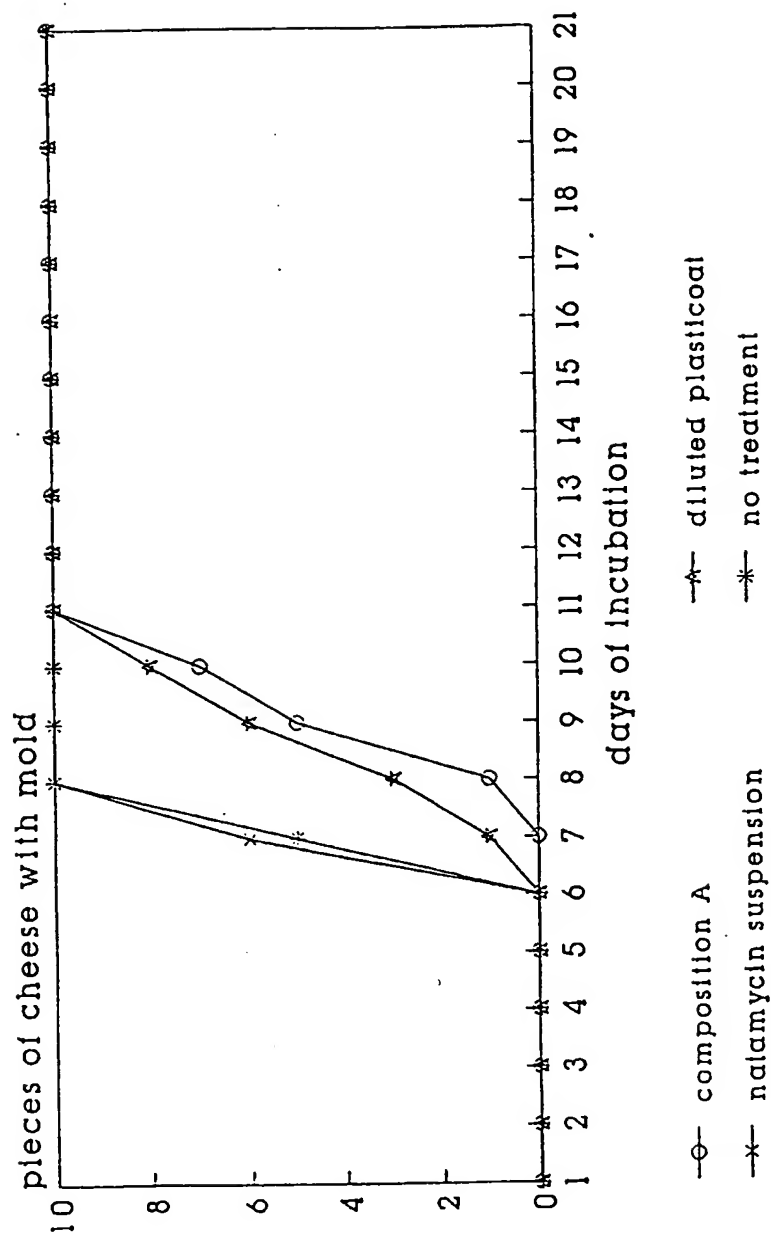


Fig. 3

## 200 PPM OF NATAMYCIN

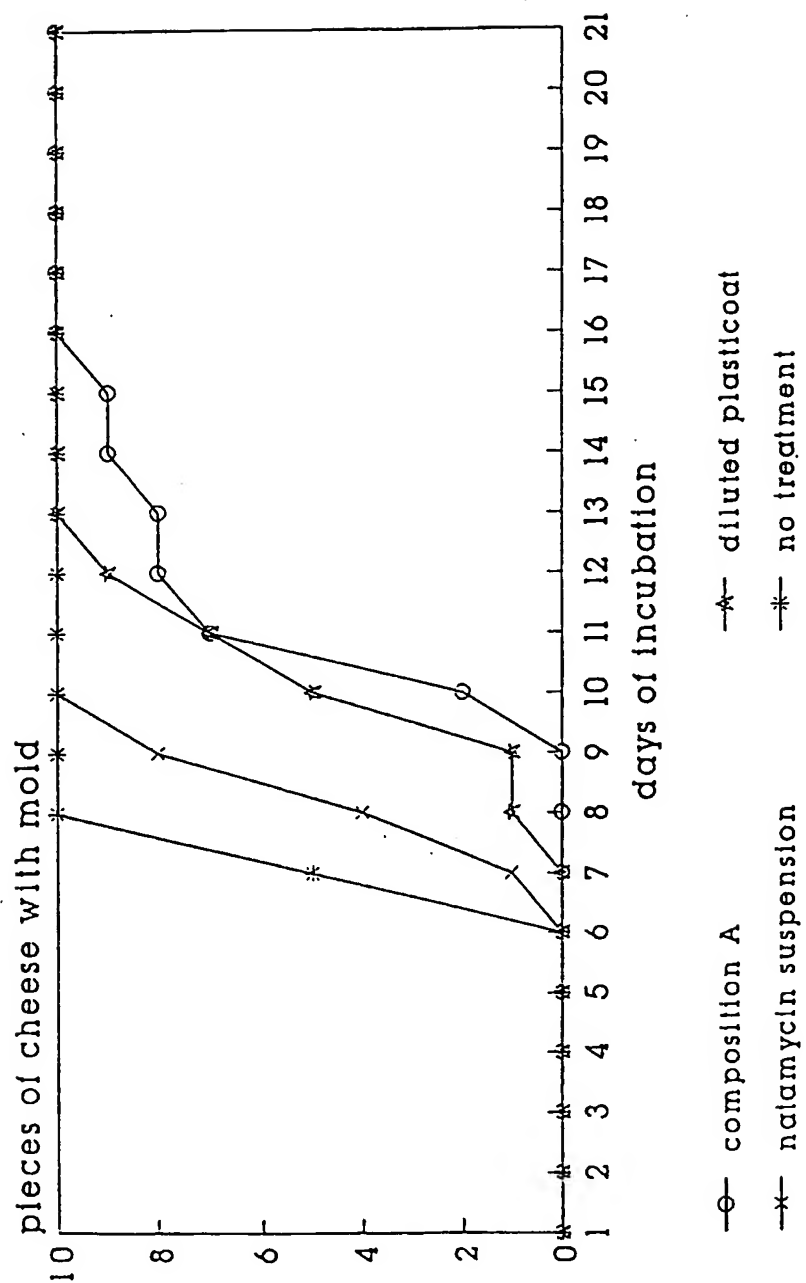


Fig. 4

## 200 PPM OF NATAMYCIN

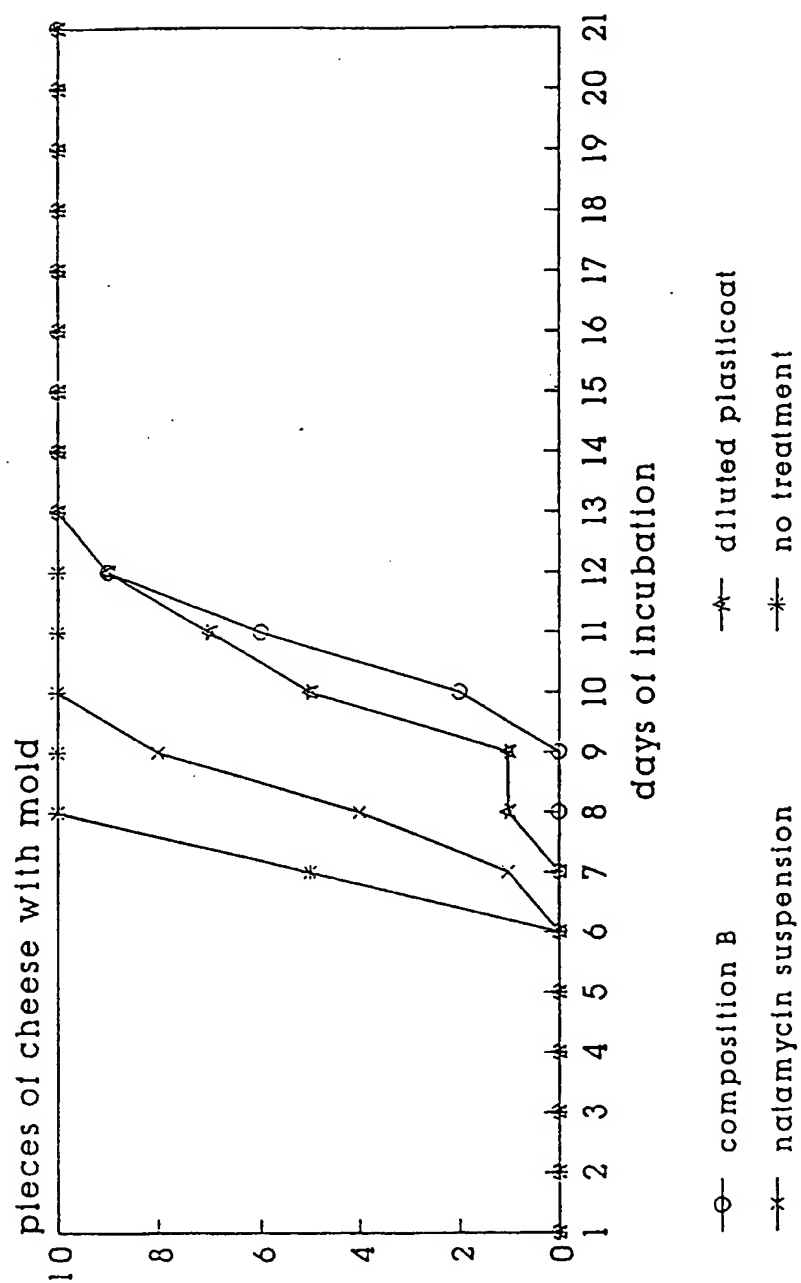
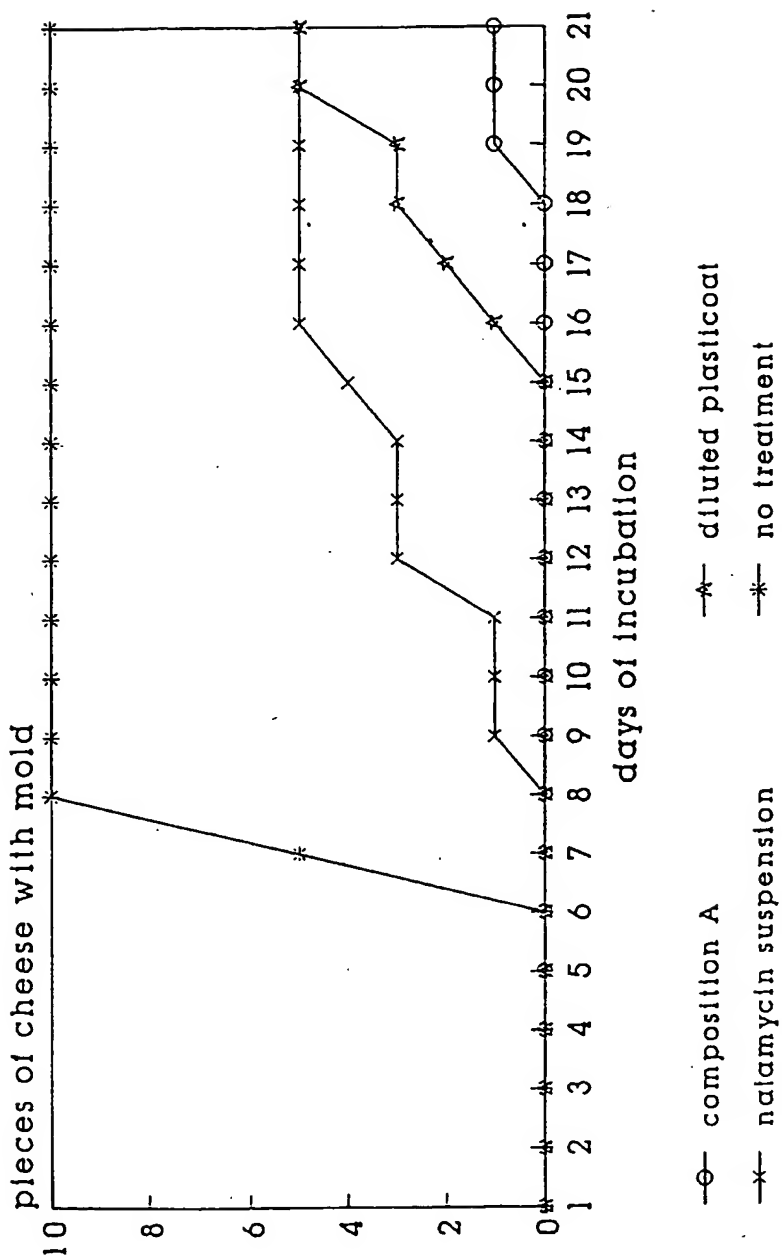


Fig. 5

# 500 PPM OF NATAMYCIN





European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 92 20 1359

### DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	BE-A-669 106 (SHELL INT. REAS. MAATSCHAPPIJ NV)  * page 20, line 25 - page 21, line 11 * * page 11, line 22 - page 13, line 30; claim 1 *	1, 4, 5, 8, 9	A23L3/3463 A23D7/00 A23C19/11 A61K7/48 A01N25/04
X	US-A-2 703 288 (WORSON)  * column 3, line 25 - line 67; claims *	1, 3, 4, 8, 9	
Y	---	2, 5	
Y	FR-A-2 371 149 (GIST BROCADES NV) * page 3, line 18 - page 4, line 26 *	2, 5	
A	DE-A-3 035 646 (FLEISCHER) * page 5; claims 1, 7-9 *	1, 2, 8, 9	
A	US-A-4 649 057 (THOMSON)  * column 2, line 61 - column 4, line 68 *	1, 3, 4, 7-9	
D, A	VOEDINGSMIDDELEN TECHNOLOGIE, vol. 18, no. 2, 24 January 1985, ZEIST NL pages 26 - 29; C.B.G. DAAMEN ET AL.: 'Voorkoming van schimmelgroei op de korst van kaas met behulp van natamycine'		TECHNICAL FIELDS SEARCHED (Int. Cl.5)  A23L A61K A01N A23C A23D A23B
The present search report has been drawn up for all claims			
Place of search THE HAGUE	Date of completion of the search 28 AUGUST 1992	Examiner LEPRETRE F.G.M.	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons  A : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			